Membranotropic Effects of Antibodies to S100 Protein in Ultralow Doses

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Two types of neurons exhibiting various reactions to application of antibodies against S100 protein in the washing solution were revealed in the nervous system of *Helix lucorum* snails. After treatment with antibodies against S100 protein the frequency of action potential generation decreased in spontaneously active B1, B3, B17, and PPa6 cells, but increased in B4 and B6 cells. The effect of antibodies against S100 was less pronounced in the solution of potentiated antibodies against this protein. After pre-exposure of ganglia in the solution of potentiated water the effect of antibodies against S100 protein decreased to a lesser extent. No significant changes were revealed in the membrane resting potential of cells. Combination treatment with antibodies and potentiated antibodies against S100 protein increased the threshold of action potential generation in B1 and B17 cells. Our results indicate that potentiated antibodies against S100 protein specifically modulate the activity of nerve cells.

Key Words: *S100 protein; antibodies against S100 protein; potentiated substances; threshold potential; identified neuron*

The family of S100 proteins was extensively studied [11,14]. S100 was mapped as a water-soluble protein. Further studies revealed that a part of this protein is bound to the membrane and may be extracted only with detergents and alcohols. Published data show that 15% S100 are present in membrane fractions, primarily in neurons and synaptosomes [12,14]. Much attention is given to S100 proteins. S100 are brain-specific proteins that bind and interact with Ca^{2+} [4,7,12]. Autoantibodies against S100 are present in the blood from patients with schizophrenia, epilepsy, multiple sclerosis, and other diseases, which indicates that these proteins play an important physiological role [5,6]. It was hypothesized that the presence of autoantibodies against S100 proteins is the major immunological sign of nervous and mental disorders [6].

Recent studies were devoted to the role of S100 proteins in biological systems and animal's behavior. New experimental, methodical, and methodological approaches to the analysis of fine mechanisms that underlie the involvement of S100 proteins in physiological activity and their use in medical practice were elaborated [3,7,11,12,14]. Antibodies against S100 proteins (AB-S100) interact with antigens and modify electrical characteristics of the neuronal membrane [4,10].

We continued our long studies of physiological activity of brain-specific S100 proteins [9]. This work was designed to evaluate the effect of pretreatment with AT-S100 in high dilutions that modify the action of substances in standard concentrations (bipathic phenomenon) [9].

MATERIALS AND METHODS

Experiments were performed on adult *Helix lucorum* snails with similar weights and sizes. Mollusks were kept in glass terrariums at room temperature, high humidity, and excess food. Before the start of experiments, snails were in the active state for no less than 2 weeks [1]. Studies were performed with identified spontaneously active neurons of snail subpharyngeal ganglion [8]. We used the isolated preparation of the central nervous system (CNS). The animals were cooled in cold water with ice for 20-30 min [2]. The measurements were performed using intracellular glass microelectrodes (5-40 MΩ) filled with 2.5 M KCl at room temperature (20-22°C). Biological potentials were recorded on a personal computer.

During microelectrode assay the following parameters of neuronal electrical activity were recorded: frequency of action potential generation (N, ppm),

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membrane resting potential, and threshold of action potential generation (threshold potential). The membrane resting potential and threshold of action potential generation were expressed in mV. The frequency of action potential generation was determined before (*Ni*) and after application of the preparation (*Ne*). The ratio between these values (%) was calculated as $n=Ne/Ni\times100\%$.

The results were analyzed by Student's *t* test and Mann-Whitney test. The significance of differences between average values characterizing neuronal activity in various series was evaluated. The data are expressed as means and standard errors ($M\pm SEM$).

We used the immune serum to nerve-specific S100 diluted with snail physiological saline (PS) in the 1:5 ratio. The lyophilized product was used in a concentration of 12 mg per 1 ml solution. Experimental solutions were prepared. Potentiated water (P-H_2O) and potentiated antibodies to S100 (PAB-S100) were prepared by the method of homeopathic potentiation and used in homeopathic dilution C3 (equivalent concentration 10^{-6} wt %, "Materia Medica Holding" Research-and-Production Company). One drop of P-H_2O or PAB-S100 was added to a flask with 5 ml snail PS. The base solution of AB-S100 was diluted with P-H_2O and PAB-S100 to obtain solutions of AB-S100 in potentiated water (P-H_2O+AB-S100) and potentiated AB-S100 (PAB-S100+AB-S100), respectively.

Membrane characteristics of identified neurons were predetermined in PS. Electrical characteristics were recorded in the solution of AB-S100, P-H_2O, or PAB-S100.

In series I the preparation of CNS was kept in PS for 20 min, mixed with AB-S100 for 30 min, and washed with PS for 30 min.

In series II the preparation of CNS was kept in PS for 20 min, mixed with PAB-S100 (20 min) and PAB-S100+AB-S100 (30 min), and washed with PS for 30 min.

In series III the preparation of CNS was kept in PS for 20 min, mixed with P-H_2O (20 min) and P-H_2O+AB-S100 (30 min), and washed with PS for 30 min.

RESULTS

Visceral and right parietal ganglia included 2 types of neurons that exhibited various reactions to application of AB-S100 in a dilution of 1:5 (base solution).

After treatment with AB-S100 the frequency of action potential generation decreased in spontaneously active B1, B3, B17, and PPa6 cells, but increased in B4, B6, and other cells (Fig. 1, *a*, *b*). Substitution of snail PS for P-H_2O and PAB-S100 was not followed by significant changes in the frequency of action potential generation in cells of both groups (Fig. 1, *a*, *b*). The effect of AB-S100 was abolished after substitution of PAB-S100 for PAB-S100+AB-S100. Under these conditions AB-S100 produced the opposite effect. The frequency of action potential generation increased in group 1 cells, but decreased in group 2 cells (Fig. 1, *a*, *b*). The effect of AB-S100 was partially abolished in P-H_2O. We revealed no significant differences in the effects of P-H_2O and PAB-S100, which was related to the wide scatter of the data. The membrane resting potential remained practically unchanged in cells of both groups. This parameter tended to undergo opposite changes. The threshold of resting potential generation significantly increased in B1 and B17 cells after treatment with AB-S100 in the solution of PAB-S100 (Fig. 2).

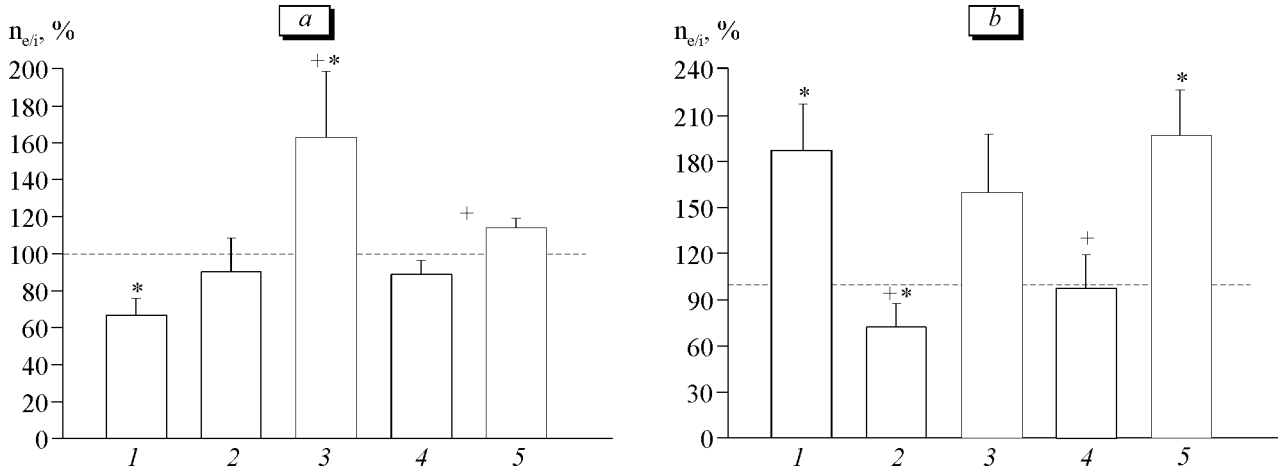

Fig. 1. Ratio between frequencies of action potential generation ($n_{e/i}$) in B1, B17 (*a*), B4, and B6 neurons (*b*) after treatment with antibodies against S100 protein (AB-S100, *1*), potentiated antibodies to S100 protein (PAB-S100, *2*), potentiated H_2O (*3*), and AB-S100 in the solution of PAB-S100 (*4*) and potentiated H_2O (*5*). $n_{e/i}=Ne/Ni$, where *Ni* and *Ne* are frequencies of action potential generation before and after application, respectively. Here and in Fig. 2: dotted line, physiological saline. $p<0.05$: *compared to physiological saline; +compared to AB-S100.

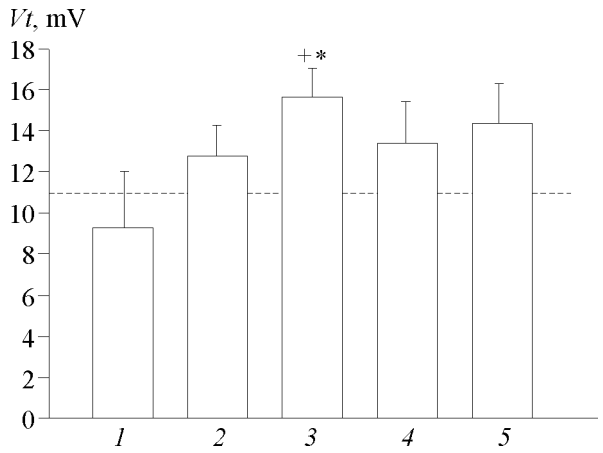

Fig. 2. Threshold of action potential generation (*Vt*) in B1 and B17 neurons after treatment with AB-S100 (*1*), PAB-S100 (*2*), potentiated H_2O (*3*), and AB-S100 in the solution of PAB-S100 (*4*) and potentiated H_2O (*5*).

Our results indicate that nerve-specific S100 proteins are associated with or involved in functional activity of membrane structures. AB-S100 produce the opposite effects on various cells. Therefore, the action of AB-S100 depends on the functional role of target membrane structures. The ability of S100 proteins to bind Ca^{2+} is important for their biological activity [4,7, 12,14]. Calcium is one of the major regulators of physiological activity in cells that acts as the secondary messenger responsible for a variety of Ca^{2+}-induced physiological changes. It cannot be excluded that AB-S100 directly affect neurotropic regulation mediated by S100 proteins [13]. Besides this, Ca^{2+} promote stabilization of the cell membrane potential. These properties of S100 proteins probably determine their polyfunctional characteristics and role in physiological functions.

The membrane concept of mechanisms underlying preventive activity of PAB-S100 seems to be most probable. Probably, PAB-S100 regulate functional activity of the calcium system via impairment of Ca^{2+} binding to proteins and direct action on membrane stabilization.

Our results show that the effect of AB-S100 is abolished in the solution of PAB-S100. The influence of AB-S100 in P-H_2O undergoes less pronounced changes. AB-S100 increase the threshold of action potential generation in B1 and B17 cells after preexposition in the solution of PAB-S100. These effects are probably related to changes in the kinetics and characteristics of Ca^{2+}-dependent processes in the neuronal membrane and influence of PAB-S100 on the membrane-stabilizing system. The data demonstrate that PAB-S100 specifically modulate properties of nerve cell membranes.

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